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Table 3 List of synthesised peptides, optimal yields and Mass values.

PEPTIDE	REDUCED YIELD %	OXIDISED YIELD %	EXPECTED MASS (Mr,Da)	OBSERVED MASS (Mr,Da)
CVID	36	35	2755	2755
R^{10}-CVID	36	40	2784	2784
$D^{9}R^{10}$-CVID	33	29	2812	2812

Example 3

Isolation and Characterisation of the CVID Gene Sequence

RNA extraction and cDNA synthesis

Two specimens of *Conus catus* were collected from Lady Elliot Island on the Queensland Great Barrier Reef. The animals were anaethesised on ice, and dissected to remove the venom duct in a region from the venom bulb to the proboscis. The ducts were sectioned, placed in a buffer containing guanidinium thiocyanate/N-lauroyl sarcosine, then emulsified with manual grinding. Poly-A tailed mRNA was extracted from the mixtures using the Pharmacia Biotech QuickPrep mRNA purification system.

Strand-1 cDNA was 3' end synthesised from the *C. catus* poly-A mRNA templates using a Not1-$d(T)_{18}$ bifunctional primer (5'-AACTGGAAGAATTCGCGGCCGCAGGAA$T_{(18)}$-3') [SEQ ID NO: 40] (Pharmacia Biotech) in conjunction with Superscript II reverse transcriptase (Gibco BRL). The resultant cDNA templates were used to manufacture double stranded cDNA using a RNaseH/DNA polymerase procedure as per the Pharmacia Biotech cDNA Timesaver

protocol. Marathon (Clontech) adaptors were then added to the 5' and 3' ends of the ds-cDNA molecules to complete the cDNA construction. A representation of a complete coneshell venom peptide cDNA molecule is shown in figure 1.

PCR derivation of CVID and related cDNA sequences

PCR was carried out on samples containing ds-cDNA from *C.catus*, the CSRD-301A primer (5' - ATCATCAAAATGAAACTGACGTC - 3') [SEQ ID NO:41], the ANCHOR primer (5' - AACTGGAAGAATTCGCGGCCGCAGGAAT - 3') [SEQ ID NO: 42] and an appropriate *Tag* polymerase (Biotech International) and buffer (25mM Mg, 100uM deoxy-nucleotides, buffered at pH 8.5) in a thermal cycler (Omnigene) at 95°C/2 mins for 1 cycle, 95°C/30 sec - 55°C/60 sec -72°C/90 sec for 35 cycles, and 72°C/10 mins for 1 cycle. This PCR produced a heterogeneous DNA product of approximately 380 bp to 500bp. Sequence analysis of clones derived from this PCR product have shown it to contain the sequence CVID as well as other related venom peptide sequences.

Cloning and sequencing of CVID

The DNA product produced from the CSRD-301A-ANCHOR driven PCR of *C. catus* cDNA was electrophoresed in low melting point agarose and excised. The DNA was extracted from the agarose on Qiagen columns, rephosphorylated with T4 DNA kinase (Progen), blunt ended with Klenow polymerase (Progen), and ligated with T4 DNA ligase (Progen) into the multiple cloning site of dephosphorylated *Sma-1* cut pUC-18 plasmid vector DNA (Pharmacia Biotech). The vector DNA was electrotransformed into Bluescript *E.coli* cells, to produce a library of clones representing the PCR product. Aliquots of the library were plated onto LB_{amp} plates, and individual clones selected and propagated overnight in TB_{amp} broth. Plasmid DNA was purified from the culture using the RPM system (BIO-101), and the PCR DNA inserts within the vector sequenced using the pUC-18 forward and reverse